19 RAPID PRESUMPTIVE TESTS (RPT)

19.1 Summary

19.1.1 Rapid presumptive tests are simple colorimetric tests that may be performed directly on blood, urine, gastric contents or liver with little or no previous sample preparation. When run with negative and positive controls, these tests are sensitive enough to detect overdoses; however since these tests are presumptive, confirmation must be performed on positive findings.

19.2 Salicylate Screen (Trinder's Test)

19.2.1 Principle: the phenolic group on salicylate reacts with ferric iron to form a violet color complex.

19.2.2 Reagents and Controls

19.2.2.1 Trinder's Reagent: dissolve 8.0 g of mercuric chloride in approximately 170 mL of dH_2O by heating. Cool the solution and add 24 mL of 1 M HCl (3.1 mL conc HCl in 100 mL dH_2O) and 5.0 g of ferric nitrate ($Fe(NO_3)_2 \cdot 9\ H_2O$). When the ferric nitrate has dissolved, dilute the solution to 200 mL with dH_2O.

19.2.2.2 0.5 mg/mL (500 mg/L) Salicylate Positive Control. Dissolve 58 mg of sodium salicylate in dH_2O and QS to 100 mL with dH_2O. Add a few drops of chloroform as a preservative.

19.2.3 Urine procedure

19.2.3.1 Add 2 drops case sample urine, negative urine and positive control into separate wells in a spot plate.

19.2.3.2 Add 2 drops Trinder's Reagent.

19.2.3.3 Compare color with positive and negative control. Violet color is positive.

19.2.4 Blood procedure

19.2.4.1 Add 1 mL case sample blood and negative control blood to separate 12 x 75 mm test tubes. Add 0.5 mL negative blood and 0.5 mL positive control to one 12 x 75 mm test tube to prepare a 250 mg/L control. Add 1 mL positive control (500 mg/L) to a separate 12 x 75 mm test tube.

19.2.4.2 Add 1 mL Trinder's reagent to each tube and vortex.

19.2.4.3 Centrifuge for 5 minutes.

19.2.4.4 Compare color with positive and negative controls. A brown to violet color in supernatant is positive.

19.3 Acetaminophen Screen (Cresol Test)

19.3.1 Principle: acetaminophen is acid hydrolyzed to p-aminophenol, which, in the presence of o-cresol, forms a blue color.

19.3.2 Reagents and Controls

19.3.2.1 Hydrochloric Acid, concentrated

19.3.2.2 Ammonium hydroxide, concentrated

19.3.2.3 6.25% Trichloroacetic Acid (TCA): in a hood, dissolve 6.25 g TCA in dH_2O and QS to 100 mL with dH_2O. Mix well.

19.3.2.4 1% o-Cresol Reagent. in a hood, add 10 mL o-cresol to 1 L volumetric flask containing dH_2O. QS to 1 L with dH_2O. Shake and allow to stand for 24 hours before use.

19.3.2.5 30 mg/L acetaminophen control: pipet 30 µL of 1 mg/mL acetaminophen stock into 1 mL blood or urine.

19.3.2.6 100 mg/L acetaminophen control: pipet 100 µL of 1 mg/mL acetaminophen stock into 1 mL blood or urine.

19.3.3 Procedure

19.3.3.1 Add 1 mL blood, urine or tissue homogenate to 16 x 100 mm test tubes. Prepare a water blank, negative, 30 and 100 mg/L positive controls.

19.3.3.2 Add 2 mL of 6.25% TCA to each tube. Vortex.

19.3.3.3 Centrifuge at 2500 rpm for 10 minutes.

19.3.3.4 Pipet 200 µL of supernatant to 12 x 75 mm test tubes.

19.3.3.5 Add 200 µL of concentrated HCl to each tube and place in a 90 - 100 ^oC heating block for 30 minutes.

19.3.3.6 Remove tubes from heating block. Allow to cool.

19.3.3.7 In a hood, add 0.5 mL cresol reagent and 0.5 mL concentrated NH_4OH

19.3.3.8 Compare unknowns against water blank, negative and positive controls. A blue color within 10 minutes is a positive result for acetaminophen.

19.4 Phenothiazine Screen (FPN)

19.4.1 Principle: phenothiazine drugs, used as tranquilizers and antihistamines, react with FPN reagent (Ferric-Perchloric-Nitric) in urine to form a colored complex. The color is observed within 10 seconds.

19.4.2 Reagents and Controls

19.4.2.1 Ferric Chloride, 5% (w/v): weigh 5 g $FeCl_3$ into a 100 mL volumetric flask. QS to volume with dH_20.

19.4.2.2 Perchloric Acid, 20% (v/v): pipet 13 mL concentrated perchloric acid in a 100 mL volumetric flask. QS to volune with dH_20.

19.4.2.3 Nitric Acid, 50% (v/v): pipet 50 mL concentrated nitric acid into a 100 mL volumetric flask. QS to volume with dH_20.

19.4.2.4 FPN Reagent: mix 5 mL 5% ferric chloride reagent , 45 mL 20% perchloric acid reagent and 50 mL 50% nitric acid reagent.

19.4.2.5 Positive Control: prepare a 100 mg/L chlorpromazine control in urine or water.

19.4.3 Urine and gastric contents procedure

19.4.3.1 Add two drops urine, positive control and negative urine control to different wells in a spot plate.

19.4.3.2 Add two drops FPN reagent and watch for color change within 10 seconds. Compare to negative and positive control. Colors fade after 10 seconds.

19.4.3.3 Colors range from pale pink, to deep violet. The color and intensity of color is dependent upon the dose and phenothiazine/metabolite present. FPN reagent may also cross-react with salicylate. Common FPN color reactions are:

- Pink: perphenazine
- Pink/orange: promethazine
- Blue: thioridazine
- Violet: chlorpromazine

19.5 Heavy Metal Screen (Reinsch Test)

19.5.1 Principle: certain heavy metals can be quickly and easily identified when ingested in acute toxic doses using the classical Reinsch Test. The Reinsch test identifies arsenic, antimony, bismuth and mercury. The test is based on the ability of metallic copper, in the presence of strong acid, to reduce selected heavy metals to their elemental form (e.g. arsenic is deposited on the copper as a visible dull black film):

$$3Cu^{0} + 2As^{+3} + HCl \rightarrow 3Cu^{+2} + 2As^{0}$$

19.5.2 Reagents and controls

19.5.2.1 Hydrochloric acid, concentrated

19.5.2.2 Copper spiral (#20 gauge) or foil strip. Wind copper around a glass rod or pencil. Clean the copper by dunking in concentrated nitric acid for a few seconds, then immediately immerse in water. The copper should be bright and shiny.

19.5.2.3 Arsenic reference solution, 1 mg/mL. Dissolve 0.132 g of arsenic trioxide in 1.0 mL of 10 N sodium hydroxide. QS to 50 mL with dH_2O. Neutralize the solution with concentrated HCl, then QS to 100 mL with dH_2O.

19.5.3 Procedure (urine, gastric contents or liver are preferred specimens)

19.5.3.1 Place clean copper spirals into separate 100 mL beakers or 125 mL Erlenmeyer flasks labeled for negative and positive controls and unknown(s).

19.5.3.2 Place 20 mL urine, approximately 10-15 g minced tissue in 20 mL dH_2O, or an aliquot of gastric contents dissolved in 20 mL dH_2O into a labeled beaker. Place 20 mL negative control urine in two separate beakers. Spike one of them with 40 µL of 1 mg/mL arsenic reference solution (final concentration, 2 mg/L).

19.5.3.3 Carefully add 4 mL concentrated HCl to each beaker.

19.5.3.4 In a hood, heat the solutions to a gentle boil for approximately 1 hour. Add 10% HCl as necessary to maintain the original volume.

19.5.3.5 After 1 hour, remove the copper coils and gently rinse with dH_2O. Compare the negative control and positive control to the unknown for the presence of a gray to black deposit.

19.5.4 Interpretation. If the positive and negative control work and the copper coils in the unknown samples are still bright in appearance, then the test can be reported as "Heavy Metals not detected (antimony, arsenic, bismuth, and mercury)." In the presence of arsenic, antimony or bismuth, the surface of the copper will be gray to black. In the

presence of mercury, the film on the copper will be light gray to silvery and become shiny on rubbing. All positives must be confirmed.

19.6 References

19.6.1 I., Sunshine, Methodology for Analytical Toxicology, CRC Press, Cleveland, OH, 1975.

19.6.2 N. W. Tietz, Fundamentals of Clinical Chemistry, W.B. Saunders, Philadelphia, PA, 1976.

19.6.3 E.C.G. Clarke, Clark's Isolation and Identification of Drugs, The Pharmaceutical Press, London, UK, 1986.